

Anatomical and Histological Characteristics of the Nervous System of the Chilean Giant Mussel, *Choromytilus chorus* (Molina 1782) (Bivalvia, Mytilidae)

Características Anatómicas e Histológicas del Sistema Nervioso del Mejillón Gigante, *Choromytilus chorus* (Molina 1782) (Bivalvia, Mytilidae)

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SUMMARY: The anatomy and histology of the nervous system in the mussel *Choromytilus chorus* were studied. Juvenile specimens of *C. chorus* and adult broodstock were collected in Laraquete Cove, Chile (37°09'S; 37°11'O). The juveniles were used for histological analysis and the adults for a macroscopic description of anatomical. The histological description was carried out by Gallego's trichrome technique. The macroscopic analysis showed that nervous system network includes three pairs of ganglia of orange color and little size (20-40 mm) (cerebral, pedal and visceral) located in the anterior, middle and posterior zone of the specimen, respectively. The histological analysis showed many type de cells inside the ganglia (neurosecretory, granulated and glial cells). The ganglia network could be involving in regulating several physiological processes in the mussels through of their neurosecretions.

KEY WORDS: Mussels; Nervous system; Histology; Neurosecretory cells; *Choromytilus chorus*.

INTRODUCTION

The family Mytilidae is an important group within the benthic marine bivalves, with a wide cosmopolitan geographical distribution (Bayne, 1976; Aldea & Valdovinos, 2005). In Chile, this family includes the species, *Semimytilus algosus* (Gould 1850), *Perumytilus purpuratus* (Lamarck 1819), *Brachidontes granulata* (Hanley 1843), *Mytilus chilensis* (Hupé 1854), *Choromytilus chorus* (Molina 1782), *Aulacomya ater* (Molina 1782) (Zagal & Hermosilla, 2001; Aldea & Valdovinos), and the recently found species *Mytilus galloprovincialis* (Tarifeño *et al.*, 2012). As in many other countries, the Chilean mussels constitute aquaculture resources of great economic importance due to their commercial extraction or their massive sea farming in suspended longline systems (Food and Agriculture Organization of the United Nations, 2014).

Choromytilus chorus stands out because of its ecological and economic importance (Moreno, 1995; Servicio Nacional de Pesca, 2016), and is distributed from Callao (Peru) (10°S) to the Strait of Magellan and the Beagle Channel

in the southeastern Pacific (54°S) (Osorio *et al.*, 2002). This mussel is gonocoric, their sex ratio is 1:1 and size at first maturity ranged from 38-40 mm (Lépez, 2005; Ruiz-Velásquez *et al.*, 2017). Beside its aquaculture relevance, *C. chorus* stands out as an excellent biological models to perform anatomical and experimental studies understanding basic mechanisms of developmental biology and fundamental processes in neuroscience, because its large average size at adult phase.

Although *C. chorus* is important in our country, it is surprising that so little is currently known about of fundamental aspects of its basic biology concerning to nervous system, both of this species as in other mytilidos. There is obviously an increasing need of do studies of basic biology in the mollusks due to strong environmental disturbances and anthropogenic to that currently they are exposed. In this context, this study describes, for the first time, the anatomy and histology of the nervous system of the Chilean giant mussel *Choromytilus chorus*, with emphasis on their role in reproductive aspects.

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MATERIAL AND METHOD

C. chorus specimens (n=10) of 30, 40 and 120 mm shell length of were collected in Laraquete Cove, BíoBío region, Chile (37°09'S; 37°11'W), in October 2011. The total shell length of each mussel was measured using a Vernier caliper (± 0.1 mm). For the histological analysis, a subset of mussels (n=3) was selected from individuals of 30 mm (n=3) and 40 mm shell length, that were classified as juveniles and adults in their first sexual maturity (Lépez). Meanwhile, individuals 120mm (n=4) were used to perform anatomic descriptions of the nervous system through visual inspection and bisection under a stereoscopic magnifying glass.

The whole specimens were fixed in a solution of 10 % formalin in sea water for 48 h. The samples were then dehydrated by immersion for one hour each in alcohols, and benzol. The samples were then embedded in paraffin, and a series of 7 μ m slides were made with a microtome (Leica Autocut 2040) in the transverse and sagittal plane. The slides sections were then stained with Gallego's trichrome staining following Gallardo (2001) and observed under an optical microscope.

RESULTS

Macroscopic analysis of the nervous system network of the mussel *C. chorus*. The nervous system of the *C. chorus* mussel showed to be bilaterally symmetrical, according to a sagittal plane with respect to the main axis of the organism. It consists of three pairs of ganglia of orange color: cerebral (CG), pedal (PG) and visceral (VG). The CG is located in the anterior area above the esophagus (near the labial palps), the PG is placed in the middle (under foot organ) and the VG is in the posterior area (on the middle area of the adductor muscle) of the individual. Each pair of ganglia is connected to each other by connective nerves and bilaterally by inter-ganglionic nerve commissures (Fig. 1).

The cerebral ganglia are connected by a long dorsal commissure known as inter-cerebral which passes over the esophagus, they have rectangular shape and are located parallel to the mouth close to the area where the lip palps originate. From each ganglion emerges the anterior pallial nerve (APN) toward the anterior region of the mantle, ramifying by all the edge of this and later joining with the posterior pallial nerve PPN (coming from the visceral ganglion) to form circumpallial nerves (CP). Branches of the buccal nerves (BN) to innervate of the labial palps are also observed from these ganglia. In addition, from each cerebral ganglion emerge two nerves in

independent form, one that connects with the pedal ganglion and another with the visceral ganglion, called cerebro-pedal connectives (CPC) and cerebro-visceral connectives (CVC), respectively (Figs. 1A-B).

The pedal ganglia are fused in the midline, although the right and left bodies are distinguishable. This pair of ganglia have twice the size of the cerebral and visceral ganglia, and are located at the base of the pedal organ (foot) attached to the first pair of anterior retractor muscles of the byssus. From each ganglion emerges the pedal nerves, which penetrate to the posterior pedal retractor muscles and enter the foot. In addition, from this nerve emerge the dorsal retractor byssus nerves (DRBN), supplying part of the posterior retractor byssus musculature and ventral retractor byssus nerves (VRBN), which supply the byssus organ and parts of the anterior and posterior retractor byssus muscles. From the anterior region of this ganglion emerge the brain-pedal connective nerve (BPCN) (Figs. 1A-1C).

The visceral ganglions are ovoid-shape are connected by a short inter-ganglionic commissure. They are located on the anterior-ventral side of the posterior adductor muscle and they are within of the gill insertion area. Ventral pallial nerves (VPN) (which have dorsal and ventral branches dorsal pallial nerves to the edge of the mantle), dorsal pallial nerves to the edge of the mantle (DPN), posterior renal nerves (RN) and posterior pedal nerves (PPN), also were observed in these ganglia. In addition, from this nerve emerge several gill nerves (GN), supplying the musculature and epithelium of the gill. From the anterior region emerges the cerebral-visceral connective nerve (CVCN) and from the posterior region a pair of nerves that go to the posterior region of the mantle (posterior pallial nerve), which join with the pallial nerves coming from the cerebral ganglia (Figs. 1A-1D).

Histological analysis of the Nervous System Network of the mussel *C. chorus*. The three ganglia and nerves are covered by a thin layer of lax connective tissue in the periphery, denominated "perineural" with a main function to protect the nerve structures. The ganglia are composed of a central nucleus called neuropilo, which presents numerous and tight nerve fibers and is surrounded by cells of different size, that together form a structure called cortex (Figs. 2-3-4). In the cortex of all ganglia, three cell types of different sizes were observed: i) large cells with abundant cytoplasm, and triangular and elongated shape of 10 mm of average size, characterized by a large nucleus (6 mm) highly euchromatinic with an evident nucleolus, resembling neurosecretory "type A" cells described by Gallardo in the mussel *Semimytilus algosus*; ii) rounded cells of scarce cytoplasm, 6-7 mm in average size, known as "granular cells", and iii) cells of small size 3-4 mm on average, known as "glial cells" (Figs. 2C).

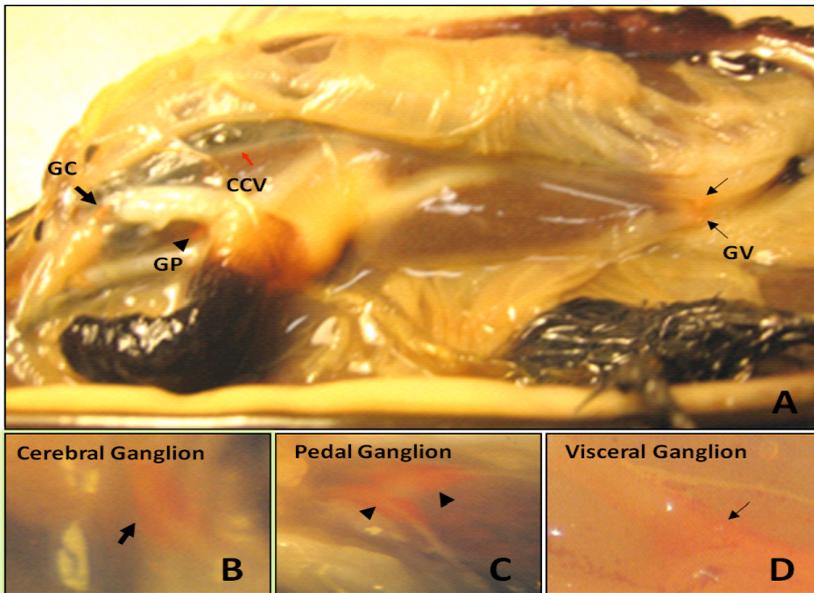


Fig. 1. Macroscopic analysis of the location of the ganglia of the mussel *Choromytilus chorus*. A) magnification of the sagittal plane that allows observing the location of the cerebral ganglia (CG), pedal ganglion (PG), visceral ganglion (VG) and the cerebral connective nerve (CCV). (B-D) further increase of the visceral ganglion.

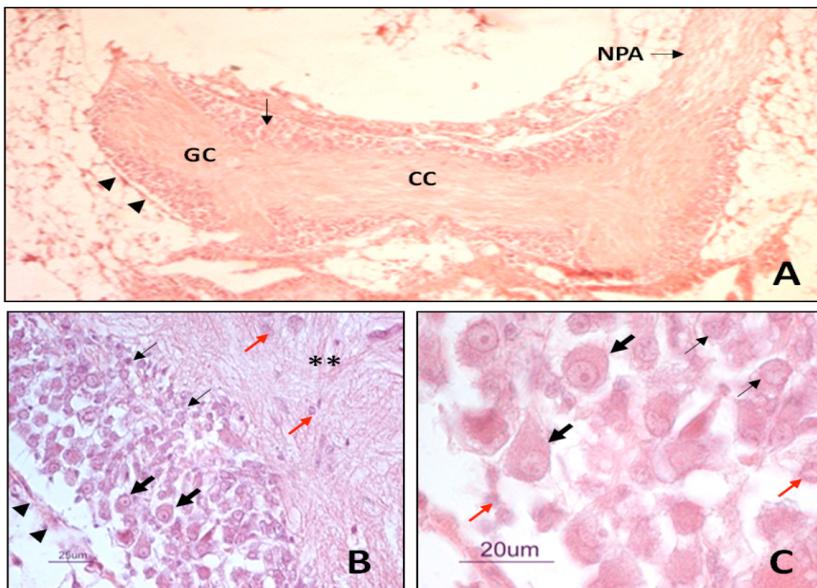


Fig. 2. Transverse section of the brain ganglia (CG) stained with Gallego's trichrome A) Low magnification shows the neural tissue surrounded by a connective capsule (arrowhead), the two ganglia joined by a fibrous commissure (CC) and the projection of the anterior pallial nerve (APN). (B-C) the ganglionic tissue shows a fibrous internal area (asterisk) and a very cellular external area, where they differentiate neurons (arrows) and medium size (small arrow) and abundant glial cells (red arrows).

The commissures, connectives and nerves have a thin layer of loose connective tissue at the periphery and bundles of fibers in the center (Figs. 2A and 4C). Cerebral ganglia presented a thick bridge of neuropilus that connects

them called the intercerebral commissure, in the anterior area of this pair of ganglia, the anterior pallial nerves emerge (Fig. 1A) and in the posterior limb are the posterior pallial connective nerves. Pedal ganglia did not show an inter-ganglionic commissure and both bodies could be seen separated by a thin perineural band (Fig. 3A). In addition, the onset of the pedal nerve that innervates the foot of these organisms, was observed (Fig. 3D).

In *C. chorus*, the visceral ganglia are associated with the adductor posterior muscle, having neurosecretory cells located preferentially in the dorsal area (Fig. 4). In *C. chorus*, individuals in juvenile phase had a lower amount of neurosecretory cells and a cytoplasm less developed than to individuals in first sexual maturity, as it was observed in juveniles of *S. algosus* (Gallardo).

DISCUSSION AND CONCLUSION

The nervous system is a key agent for coordinating and regulating physiological functions in the invertebrates (e.g., reproduction, feeding, metabolism and behavioral) by connection between the external environment and internal processes in response to environmental variations (temperature and salinity) (Mathieu *et al.*, 1991). The perception of external stimulation in molluscs is captured and channeled by cells and/or highly specialized organs (thermal receptors, statoreceptors, and paleal sensory organ), located mainly on the edge of the bivalve mantle (Zaiko, 2003) which transmit the information from the periphery toward nerve centers (ganglia), that in response synthesize and release different types of chemical messengers or neurosecretions for controlling the activity of target tissues as a specific response (Mathieu *et al.*).

In this study, the juveniles and adults stages presented a nervous network fully formed, and their anatomy

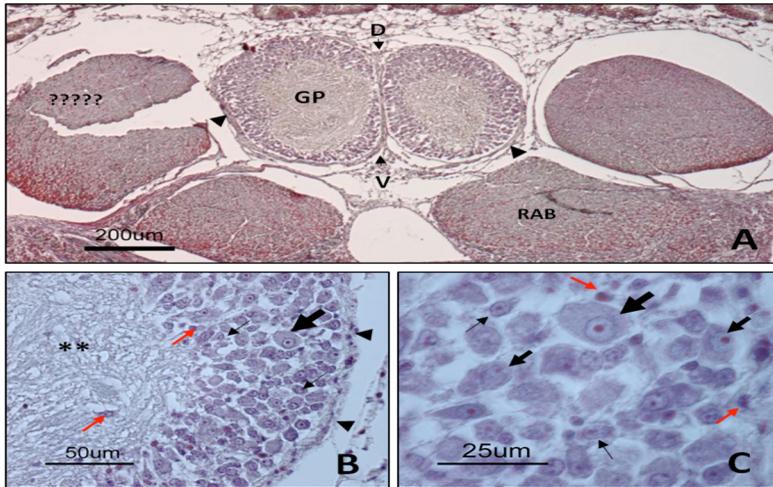


Fig. 3. Transverse section of the pedal ganglia (PG) stained with Gallego's trichrome. A) Low magnification shows the neural tissue surrounded by a conjunctive capsule (arrowhead), the two ganglia are close to the anterior retractor muscles of the byssus (RAB) and the retractor muscles of the foot (RFM). It highlights the differential distribution of the cellular component of the cortex in the ventral (V) and dorsal region (D). (B-C) The ganglion tissue shows a fibrous internal zone (asterisks) and a very cellular external zone, where we can differentiate three types of neurons; large (medium arrows), small (small arrows), and abundant glial cells (red arrows).

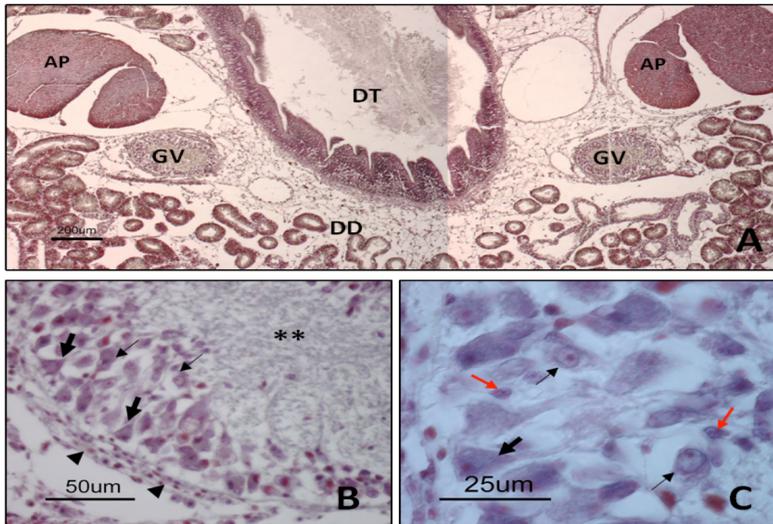


Fig. 4. Transverse section of visceral ganglia stained with Gallego's trichrome. A) Low magnification shows the neural tissue constituted by two small ganglia (VG), close to digestive structures and the posterior abductor muscles (AP). (BC) The ganglionic tissue shows a fibrous internal zone (asterisks) and a very cellular external zone, where large neurons of a pyramid-shaped (thick arrows) and small with rounded appearance (medium arrow) are observed, in addition to abundant glial cells (red arrows). Capsule of the connective tissue (arrow head). Abbreviations: S: stomach, DD: digestive diverticulum.

and spatial distribution were similar in both stages. In bivalve, the formation of the nervous system occurs early during its ontogenetic development, the appearance of the first neurons that constituting the nerve ganglia occurs in trochophore larva stage (Croll & Dickinson, 2004). *Choromytilus chorus*

has a nervous network system similar in form and structure to other species of the family Mytilidae (Bayne; Gallardo; Zaixso, 2003); *Mytilus edulis* (Gosling 1992), *Mytilus californianus* (Smith, 1997), *Semimytilus algosus* and *Aulacomya ater*. However, in *C. chorus* as in *A. ater* it was observed that both the cerebral-pedal and cerebral-visceral connective nerves are not fused to a common axis, but they emerge after the origin of the cerebral ganglia as has been described for others species of the genus *Mytilus* (List, 1902; Field, 1922).

Choromytilus chorus has ganglion and nerve structures that are innervating important organs, as such as the pedal ganglion with the anterior muscles of the byssus retractor and muscles of the foot. Studies of removal of nerve centers in *Mytilus edulis* showed that the retraction of the foot is controlled almost entirely by reflex arches from the pedal ganglion, although in other species seems to be involved the cerebral ganglion (Bayne). Meanwhile, the visceral ganglion controls the cardiomodulation (Kodirov, 2011) and movement of the valves, mantle, siphons and gills in mollusks (Bayne). The brain ganglion seems to control the gonadic tissue through neurosecretions and nerve endings and the fibers of the byssus (Mathieu *et al.*).

The histological analysis showed the presence of at least three different cell types in the ganglions: granular, glial and neurosecretory. This latter, showed anatomical feature (abundant cytoplasm, core euromatinic, nucleolus evident, size and shape) similar to that reported for neurosecretory cells of other species of the family Mytilidae (Gallardo; Zaixso). The neurosecretory cells may also be involved in regulating various physiological processes in this mussel, for example, the neurosecretion in lamellibranches has been associated with the control of the gonadal mitosis, glycogen metabolism, ciliary activity, growth, mobility, responses to environmental stress (e.g. salinity and temperature) and in particular on changes related to the ontogenetic development of this individuals (Mathieu *et al.*; Carroll & Catapane, 2007).

The close correlation between the annual neurosecretory cycle of the “type A1 cells” and the gametogenic cycle suggests a possible role of these cells in the control of the reproduction in mytilids; secretory material is accumulated in the cerebral ganglia (contain 75 % of the neurosecretory cells) during gametogenesis and then evacuated from the cells when the gametes become fully mature (Mathieu *et al.*). The results of this study suggest that the secretion of this type of cells would be regulating reproductive processes in *C. chorus*, because individuals in juvenile phase had a lower amount of neurosecretory cells (type A1 cells), specially in the cerebral ganglion, and a cytoplasm less developed compared to individuals in first sexual maturity, as has been observed by in juveniles of *S. algosus* Gallardo.

The neurosecretory cells presents in the nervous ganglion of *C. chorus* could have peptides analogous to the vertebrate, as has been established in several species of mollusks (Bayne; Mathieu *et al.*). For example, in several species of bivalves has been observed the presence of peptides, as such as insulin-like IGFs in the cerebral ganglia of *Mytilus edulis* (Kellner-Cousin *et al.*, 1994), somatostatin GHIH and cholecystokinin CCK in the cerebral ganglia of *Mytilus edulis* (Mathieu & Van Minnen, 1989), melanocyte stimulating hormone HSM in the pedal ganglia (Mathieu *et al.*), gonadotropin-releasing hormone GnRH in *Patinopecten yessoensis* (Pazos & Mathieu, 1999; Nakamura *et al.*, 2007), FMRFamide in *Mytilus edulis* (Favrel *et al.*, 1998), APGWamide in *Placopecten magellanicus* (Smith *et al.*) and serotonin (5-HT) in *Patinopecten yessoensis* and *Mytilus edulis* (Matsutani & Nomura, 1986).

In conclusion, the results of this study have shown that the anatomical and histological characterizations of the nervous system of *C. chorus* are similar to the descriptions made for other species of mytilids, differing only in the absence of a common axis of the connective tissues cerebral-visceral nerves and brain-pedals, just as happens in *Aulacomya ater*. However, it is essential to continue research in this area, especially in studies relating to the receptors and neurotransmitters that allow understanding the internal mechanisms and signaling ways that are related to the environment.

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RESUMEN: Se estudió la anatomía e histología del sistema nervioso en el coro *Choromytilus* del mejillón. Se recolectaron especímenes juveniles de *C. chorus* y reproductores adultos en Laraquete Cove, Chile (37 ° 09'S, 37 ° 11'O). Los especímenes juveniles se utilizaron para el análisis histológico y los adultos para una descripción macroscópica de anatómica. La descripción histológica se realizó mediante la técnica de tricómico de Gallego. El análisis macroscópico mostró que la red del sistema nervioso incluye tres pares de ganglios de color naranja y poco tamaño (20-40 mm) (cerebral, pedal y visceral) localizados en la zona anterior, media y posterior de la muestra, respectivamente. El análisis histológico mostró muchos tipos de células dentro de los ganglios (células neurosecretoras, granuladas y gliales). La red de ganglios podría estar involucrada en la regulación de varios procesos fisiológicos en los mejillones a través de sus neurosecreciones.

PALABRAS CLAVE: Mejillones; Sistema nervioso; Histología; Células neurosecretoras; Coro *Choromytilus*.

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